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(54) Title: CELLULAR RECEPTOR MUTATIONS PREDICTED BY MULTIPLE-SEQUENCE-ALLIGNMENT

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                                  *
gpr8_human    255-RRKVTVLVLVVLAVCLLCWTPFHLAS-280
                        E
a1ab_human    289-EKKAAKTLGIVVGMFILCWLPFFIAL-314
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A

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                   *                    *
gpr8_human    117-CKLVLAVDHYNIFSSIYFLAVMSVDRY-143
                              A
ag2r_human    101-CKIASASVSFNLYASVFLLTCLSIDRY-127
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(57) Abstract: The invention relates to methods of predicting mutations that alter the activity of a receptor in a desired manner. The methods utilize multiple sequence alignment and phylogenetic profiling to identify the relatives of a given receptor that are most likely to provide useful data allowing prediction of sites to mutate in the given receptor. The methods provided are applicable to any type of receptor, and are particularly well suited for predicting sites to mutate in order to alter the activities of the so-called orphan receptors, for which no agonists are known.

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METHOD OF PREDICTING MUTATIONS

Claims of **WO0127632**

CLAIMS1. A method of predicting a site for mutation of a first cellular receptor wherein the mutation alters the activity of the first cellular receptor, comprising the stepsof :
(a) performing a multiple sequence alignment of the first cellular receptor with other cellular receptors in the same receptor family;
(b) allocating the first cellular receptor to a receptor sub-family; and
(c) selecting an amino acid residue of the first cellular receptor for mutation, wherein the amino acid residueisanalogous to a residue, the mutation of which is known to cause altered activity of a second cellular receptor, whereby a site for mutation of the first cellular receptor is predicted.

2. The method of claim 1 wherein said first and second cellular receptors are cell surface receptors.

3. The method of claim 1 wherein said first and second cellular receptors are Gprotein coupled receptors.

4. The method of claim 3 wherein said first G protein-coupled receptor is an orphan receptor.

5. The method of claim 3 wherein step (b) is performed by phylogenetic profiling.

6. The method of claim 3 wherein the amino acid residue selected for mutation is an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of a humana, B adrenoceptor.

7. The method of claim 3 wherein the amino acid residue selected for mutation is a conserved asparagine residue analogous to N111 in transmembrane region 3 of a type1A angiotensin II receptor.

8. The method of claim 3 wherein the amino acid selected for mutation is analogous to an amino acid known to cause altered activity in a close relative of the first G protein coupled receptor.

9. The method of claim 3 wherein the second G protein-coupled receptor is in the same sub-family as the first G protein-coupled receptor.

10. The method of claim 1 wherein the mutation causes the receptor to become constitutively activated.

11. A method of obtaining a mutant of a first cellular receptor, wherein the mutant has altered activity as compared to wild type first cellular receptor, comprising the steps of :
(a) performing a multiple sequence alignment of the first cellular receptor with other cellular receptors in the same family;
(b) allocating the first cellular receptor to a cellular receptor sub-family;
(c) selecting an amino acid residue of said first cellular receptor for mutation, wherein the selected amino acid residue is analogous to a residue of a second cellular receptor, the mutation of which is known to cause altered activity of said second cellular receptor;
(d) mutating the selected amino acid residue of the cellular receptor; and
(e) expressing the mutated cellular receptor in a cell.

12. The method of claim11 wherein said first and second cellular receptors are cell surface receptors.

13. The method of claim 11 wherein said first and second cellular receptors are G-protein coupled receptors.

14. The method of claim 13 wherein the first G protein-coupled receptor is an orphan receptor.

15. The method of claim 11 wherein step (b) is performed by phylogenetic profiling.

16. The method of claim 13 wherein the amino acid residue selected for mutation is an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of the humanalB adrenoceptor.

17. The method of claim 13 wherein the amino acid residue selected for mutation is a conserved asparagine residue analogous toN 111 in transmembrane region 3 of a type I A angiotensin II receptor.

18. The method of claim 11 wherein the amino acid selected for mutation is analogous to an amino acid known to cause altered activity in a close relative of the first cellular receptor.

19. The method of claim11 wherein the second cellular receptor is in the same sub family as the first cellular receptor.

20. The method of claim 11 wherein the mutation causes the receptor to become constitutively activated.

21. A method of predicting a site for mutation of a first cellular receptor wherein the mutation alters the activity of said first cellular receptor, said method comprising the stepsof :
(a) performing a multiple sequence alignment of the cellular receptor with other cellular receptors in the same receptor family;
(b) allocating said cellular receptor to a receptor sub-family;
(c) determining whether mutant data are available for a member of the same subfamily as said cellular receptor; wherein if mutant data are available, then selecting an amino acid residue for mutation, wherein the selected amino acid residue is analogous to a residue, the mutation of which is known to cause altered activity of said member of the same sub-family as the cellular receptor,

whereby a site for mutation of said cellular receptor is predicted.

22. The method of claim 21 wherein said first cellular receptor is a cell surface receptor.

23. The method of claim 21 wherein said first cellular receptor is a G-protein coupled receptor.

24. The method of claim 23 wherein the first G protein-coupled receptor is an orphan receptor.

25. The method of claim 23 wherein the amino acid residue selected for mutation is an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of the humanalB adrenoceptor.

26. The method of claim 23 wherein the amino acid residue selected for mutation is a conserved asparagine residue analogous toN 111 in transmembrane region 3 of a type1 A angiotensin II receptor.

27. The method of claim 21 wherein step (b) is performed by phylogenetic profiling.

28. The method of claim 21 wherein the amino acid selected for mutation is analogous to an amino acid known to cause altered activity in a member of the same sub family as said first cellular receptor.

29. The method of claim 21 wherein the second cellular receptor is in the same sub family as the first cellular receptor.

30. The method of claim 21 wherein the mutation causes the receptor to become constitutively activated.

31. A method of predicting a site for mutation of a first G protein-coupled receptor wherein the mutation alters the activity of the G protein-coupled receptor, comprising the stepsof :
(a) performing a multiple sequence alignment of said first G protein-coupled receptor with other G protein-coupled receptors;
(b) allocating said first G protein-coupled receptor to a G protein-coupled receptor sub-family;
(c) determining whether mutant data are available for a member of the same subfamily as said first G protein-coupled receptor; wherein if mutant data are available then selecting an amino acid residue for mutation, wherein the selected amino acid residue is analogous to a residue whose mutation is known to cause altered activity of a close relative of the G protein-coupled receptor; and wherein if mutant data are not available then selecting an amino acid residue for mutation by identifying an amino acid residue selected from the group consisting of a conserved asparagine residue analogous toN111 in transmembrane region 3 of a type 1A angiotensin II receptor and an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of the humana, B adrenoceptor; andwhereby a site for mutation of a G protein-coupled receptor is predicted.

32. The method of claim 31 wherein said first G protein-coupled receptor is an orphan receptor.

33. The method of claim 31 wherein step (b) is performed by phylogenetic profiling.

34. The method of claim 31 wherein the mutation causes the receptor to become constitutively activated.

35. A method of obtaining a mutant of afirstcellular receptor, wherein the mutant has altered activity as compared to wild type of said cellular receptor, comprising the steps of :
(a) performing a multiple sequence alignment of the first cellular receptor with other cellular receptors in the same family;
(b) allocating the cellular receptor to a cellular receptor sub-family;
(c) determining whether mutant data are available for a member of the same subfamily as said cellular receptor; wherein if mutant data are available then selecting an amino acid residue for mutation, wherein the selected amino acid residue is analogous to a residue whose mutation is known to cause altered activity of a member of the same sub-family as said cellular receptor;
(d) mutating the selected amino acid residue of the cellular receptor; and
(e) expressing the mutated cellular receptor in a cell.

36. The method of claim 35 wherein said first and second cellular receptor is a cell surface receptor.

37. The method of claim 35 wherein said first cellular receptor is a G-protein coupled receptor.

38. The method of claim 37 wherein the first G protein-coupled receptor is an orphan receptor.

39. The method of claim 37 wherein the amino acid residue selected for mutation is an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of the humanalB adrenoceptor.

40. The method of claim 37 wherein the amino acid residue selected for mutation is a conserved asparagine residue analogous toN111 in transmembrane region 3 of a type1A angiotensin1l receptor.

41. The method of claim 35 wherein step (b) is performed by phylogenetic profiling.

42. The method of claim 35 wherein the amino acid selected for mutation is analogous to an amino acid known to cause altered activity in a member of the same sub family as the first cellular receptor.

43. The method of claim 35 wherein the second cellular receptor is in the same sub family as the first cellular receptor.

44. The method of claim 35 wherein the mutation causes the receptor to become constitutively activated.

45. A method of obtaining a mutant of a first G protein-coupled receptor, wherein the mutant has altered activity as compared to wild type of said G protein-coupled receptor, comprising the stepsof :
(a) performing a multiple sequence alignment of the first G protein-coupled receptor with other G protein-coupled receptors;
(b) allocating the G protein-coupled receptor to a G protein-coupled receptor subfamily;
(c) determining whether mutant data are available for a member of the same subfamily as the first G protein-coupled receptor; wherein if mutant data are available then selecting an amino acid residue for mutation, wherein the selected amino acid residue is analogous to a residue whose mutation is known to cause altered activity of a member of the same sub-family as said first G protein-coupled receptor; and wherein if mutant data are not available then selecting an amino acid residue for mutation by identifying an amino acid residue selected from the group consisting of a conserved asparagine residue analogous toN111 in transmembrane region 3 of a type1A angiotensin II receptor and an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of thehuman) B adrenoceptor;
(d) mutating the selected amino acid residue of the G protein-coupled receptor; and
(e) expressing the mutated G protein-coupled receptor in a cell.

46. The method of claim 45 wherein the first G protein-coupled receptor is an orphan receptor.

47. The method of claim 45 wherein step (b) is performed by phylogenetic profiling.

48. The method of claim 45 wherein the mutation causes the receptor to become constitutively activated.

49. The method of claim 45 wherein the amino acid residue selected for mutation is an amino acid residue that is 16 residues N-terminal to a conserved proline, wherein the conserved proline is analogous to P309 in transmembrane region 6 of the humana, B adrenoceptor.

50. The method of claim 45 wherein the amino acid residue selected for mutation is a conserved asparagine residue analogous toN111 in transmembrane region 3 of a type1A angiotensin II receptor.

51. The method of claim 45 wherein the amino acid selected for mutation is analogous to an amino acid known to cause altered activity in a close relative of the first cellular receptor.

52. The method of claim 45 wherein the second cellular receptor is in the same sub family as the first cellular receptor.

53. A mutated GPR8 receptor comprising altered activity as compared to a wild type GPR8 receptor, wherein the GPR8 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 124 from aspartate to alanine, a mutation at amino acid 127 from asparagine to alanine and a mutation at amino acid 259 from threonine to glutamate. 54. A mutated GPR7 receptor comprising altered activity as compared to a wild type GPR7 receptor, wherein the GPR7 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 116 from aspartate to alanine, a mutation at amino acid 119 from asparagine to alanine and a mutation at amino acid 250 from threonine to glutamate.

55. A mutatedGPR10 receptor comprising altered activity as compared to a wild type GPR10 receptor, wherein the GPR10 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 244 from tyrosine to glutamate and a mutation at amino acid 247 from valine to glutamate.

56. A mutated GPR1 receptor comprising altered activity as compared to a wild type GPR1 receptor, wherein the GPR1 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 176 from arginine to alanine, a mutation at amino acid 245 from phenylalanine to glutamate, and a mutation at amino acid 120 from asparagine to alanine.

57. A mutatedGPR17 receptor comprising altered activity as compared to a wild type GPR17 receptor, wherein the GPR17 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 114 from asparagine to alanine and a mutation at amino acid 234 from valine to glutamate.

58. A mutated GPR4 receptor comprising altered activity as compared to a wild type GPR4 receptor, wherein the GPR4 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 100 from asparagine to alanine and a mutation at amino acid 223 from lysine to glutamate.

59. A mutated GPR15 receptor comprising altered activity as compared to a wild type.

GPR15 receptor, wherein the GPR15 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 116 from asparagine to alanine and a mutation at amino acid 240 from isoleucine to glutamate.

60. A mutated GPR20 receptor comprising altered activity as compared to a wild type GPR20 receptor, wherein the GPR20 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 133 from asparagine to alanine and a mutation at amino acid 240 from methionine to glutamate.

61. A mutated HM74 receptor comprising altered activity as compared to a wild type HM74 receptor, wherein the HM74 receptor comprises a mutation selected from the

group consisting of a mutation at amino acid110 from asparagine to alanine and a mutation at amino acid 230 from isoleucine to glutamate.

62. A mutated OGR1 receptor comprising altered activity as compared to a wild type OGR1 receptor, wherein the OGR1 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 104 from asparagine to alanine and a mutation at amino acid 227 from glutamine to glutamate.

63. A mutatedEBI2 receptor comprising altered activity as compared to a wild type EBI2 receptor, wherein theEBI2 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 114 from asparagine to alanine and a mutation at amino acid 243 from leucine to glutamate.

64. A mutated BONZO receptor comprising altered activity as compared to a wild type BONZO receptor, wherein the BONZO receptor comprises a mutation selected from the group consisting of a mutation at amino acid 112 from asparagine to alanine and a mutation at amino acid 230 from leucine to glutamate.

65. A mutatedRDC1 receptor comprising altered activity as compared to a wild type RDC1 receptor, wherein theRDC1 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 127 from asparagine to alanine and a mutation at amino acid 251 from arginine to glutamate.

66. A mutated 015218 receptor comprising altered activity as compared to a wild type 015218 receptor, wherein the 015218 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 136 from asparagine to alanine and a mutation at amino acid 257 from cysteine to glutamate.

67. A mutated H963 receptor comprising altered activity as compared to a wild type H963 receptor, wherein the H963 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 97 from asparagine to alanine and a mutation at amino acid 222 from leucine to glutamate.

68. A mutated GPR30 receptor comprising altered activity as compared to a wild type GPR30 receptor, wherein the GPR30 receptor comprises a mutation selected from the group consisting of a mutation at amino acid 140 from asparagine to alanine and a mutation at amino acid 258 from leucine to glutamate.

69. A mutated GPR2 receptor comprising altered activity as compared to a wild type GPR2 receptor, wherein the GPR2 receptor comprises a mutation at amino acid 238 from leucine to glutamate.

70. A mutated GPR5 receptor comprising altered activity as compared to a wild type GPR5 receptor, wherein the GPR5 receptor comprises a mutation at amino acid 224 from valine to glutamate.

71. A mutatedGPR13 receptor comprising altered activity as compared to a wild type GPR13 receptor, wherein the GPR13 receptor comprises a mutation at amino acid 230 from isoleucine to glutamate.

72. A mutatedGPR18 receptor comprising altered activity as compared to a wild type GPR18 receptor, wherein the GPR18 receptor comprises a mutation at amino acid 231 from isoleucine to glutamate.

73. A mutated GPR21 receptor comprising altered activity as compared to a wild type GPR21 receptor, wherein the GPR21 receptor comprises a mutation at amino acid 251 from alanine to glutamate.

74. A mutated GPR22 receptor comprising altered activity as compared to a wild type GPR22 receptor, wherein the GPR22 receptor comprises a mutation at amino acid 312 from phenylalanine to glutamate.

75. A mutated GPR25 receptor comprising altered activity as compared to a wild type GPR25 receptor, wherein the GPR25 receptor comprises a mutation at amino acid 242 from leucine to glutamate.

76. A mutated GPR31 receptor comprising altered activity as compared to a wild type GPR31 receptor, wherein the GPR31 receptor comprises a mutation at amino acid 221 from glutamine to glutamate.

77. A mutated GPR38 receptor comprising altered activity as compared to a wild type GPR38 receptor, wherein the GPR38 receptor comprises a mutation at amino acid 297 from valine to glutamate.

78. A mutated GPR39 receptor comprising altered activity as compared to a wild type GPR39 receptor, wherein the GPR39 receptor comprises a mutation at amino acid 282 from isoleucine to glutamate.

79. A mutated GPR40 receptor comprising altered activity as compared to a wild type GPR40 receptor, wherein the GPR40 receptor comprises a mutation at amino acid 223 from alanine to glutamate.

80. A mutated GPR41 receptor comprising altered activity as compared to a wild type GPR41 receptor, wherein the GPR41 receptor comprises a mutation at amino acid 224 from alanine to glutamate.

81. A mutated GPR42 receptor comprising altered activity as compared to a wild type GPR42 receptor, wherein the GPR42 receptor comprises a mutation at amino acid 224 from alanine to glutamate.

82. A mutated GPR43 receptor comprising altered activity as compared to a wild type GPR43 receptor, wherein the GPR43 receptor comprises a mutation at amino acid 221 from valine to glutamate.

83. A mutated MGR receptor comprising altered activity as compared to a wild type MGR receptor, wherein the MGR receptor comprises a mutation at amino acid 263 from tyrosine to glutamate.

84. A method of identifying a compound which modulates the activity of a receptor as claimed in any one of claims 53-83, the method comprising
a) contacting a candidate compound with said receptor, and
b) determining activity of said receptor in the presence of said compound,
wherein a difference in receptor activity in the presence and absence of said candidate compound is indicative of compound modulation.

85. The method of claim 84, wherein said compound is further determined to be an inverse agonist, partial agonist or an agonist of said receptor activity.